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Faull, Olivia K ; Subramanian, Hari H ; Ezra, Martyn ; Pattinson, Kyle T S

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# The midbrain periaqueductal gray as an integrative and interoceptive neural structure for breathing

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## ABSTRACT

The periaqueductal gray (PAG) plays a critical role in autonomic function and behavioural responses to threatening stimuli. Recent evidence has revealed the PAG's potential involvement in the perception of breathlessness, a highly threatening respiratory symptom. In this review, we outline the current evidence in animals and humans on the role of the PAG in respiratory control and in the perception of breathlessness. While recent work has unveiled dissociable brain activity within the lateral PAG during perception of breathlessness and ventrolateral PAG during conditioned anticipation in healthy humans, this is yet to be translated into diseases dominated by breathlessness symptomatology, such as chronic obstructive pulmonary disease. Understanding how the substructures of the PAG differentially interact with interoceptive brain networks involved in the perception of breathlessness will help towards understanding discordant symptomatology, and may reveal treatment targets for those debilitated by chronic and pervasive breathlessness.

## 1. Introduction

Respiration is an essential facet in sustaining all forms of life, whereby prolonged respiratory failure leads to death. In humans and other species, breathing is precisely controlled to maintain homeostasis, adapting to activities and environments to maintain sufficient oxygen supply to tissues (Kreuzer, 1982). Yet, as humans, our breathing is so much more than merely an end-interface with homeostasis. The monitoring and maintenance of our breathing are reliant on accurate interoception, or the sensing of the physiological state of our body (Craig, 2002). Specifically, breathing occupies a unique liminal space within interoception, where both sensory and motor integration swap between subconscious monitoring and reflexive control, to conscious perception and voluntary control. Breathing also simultaneously bridges interoception and exteroception, amplifying the transmission of selective sensory cues from our surrounding environment, such as smell, temperature and humidity.

Thus, when breathing is considered in light of both its physiological and interoceptive properties, two important points arise: 1) That brainstem autonomic networks need to intricately interface with higher order sensation and motor control networks in the brain for both conscious and subconscious ventilatory monitoring and action selection; and 2) The sensory inputs associated with labored, unsatisfied,

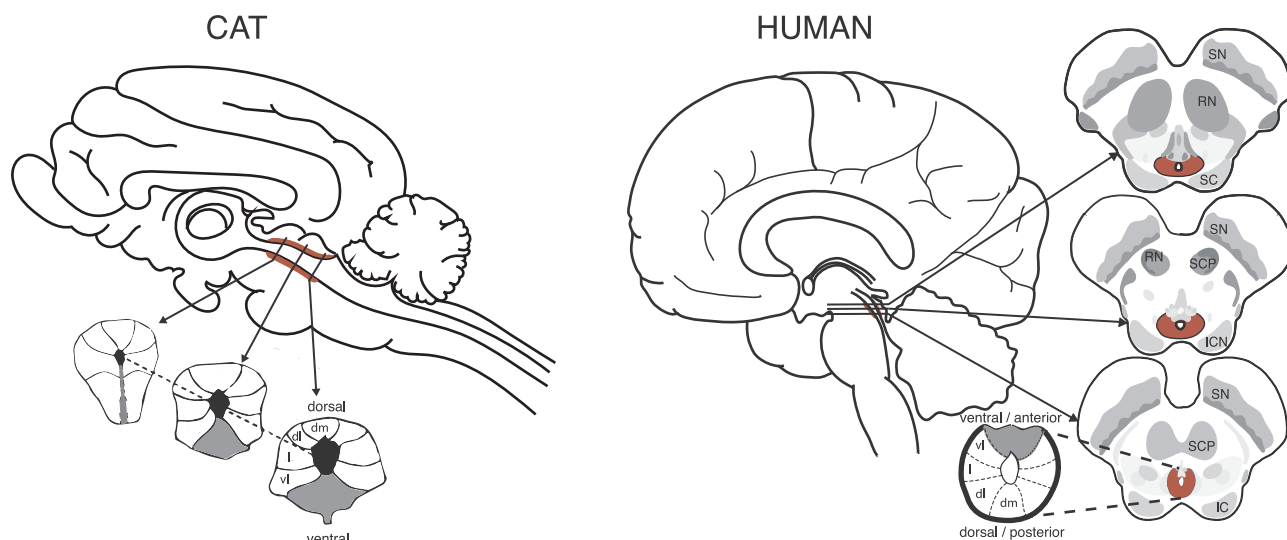
unexpected or uncontrolled breathing may induce a unique form of debilitating interoceptive threat, expressed as breathlessness. In this review, we propose that substructures of the midbrain periaqueductal gray (PAG) may integrate the perception of threat with breathing control mechanisms, and we will outline the work from animals through to humans to support our hypothesis.

### 1.1. Overview of the structure of the PAG in animals and humans

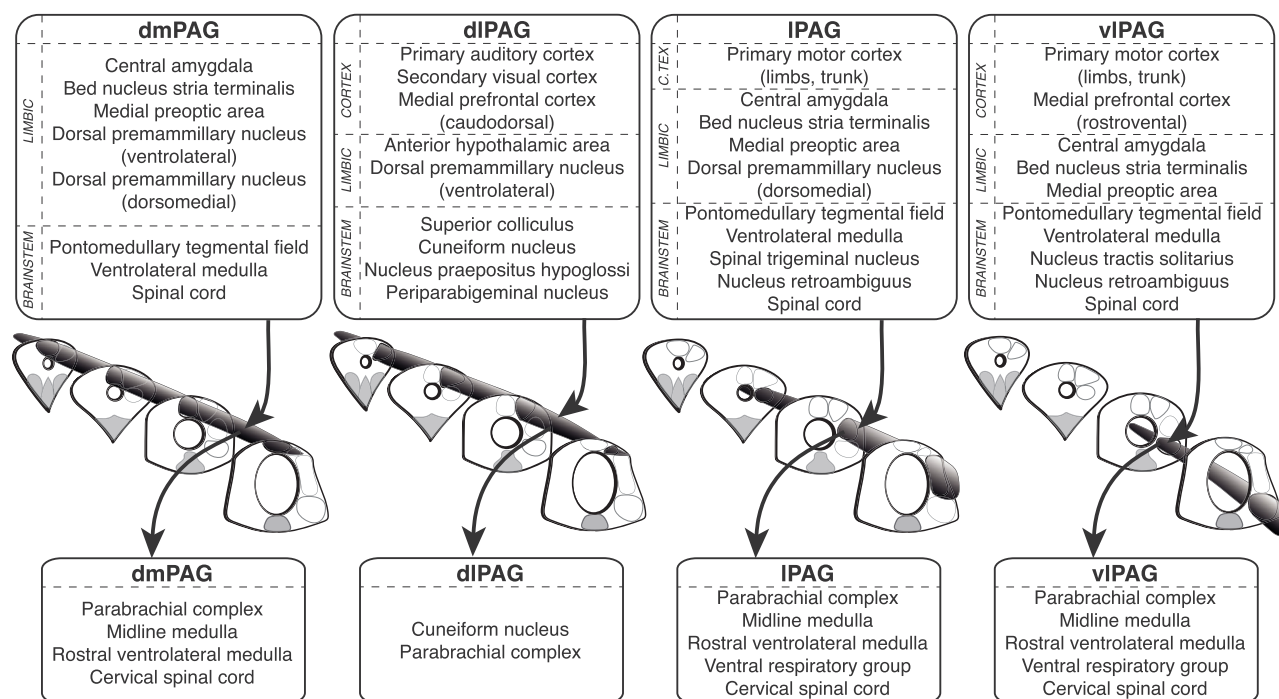
The PAG constitutes a specific portion of the ventricular gray matter. It surrounds the mesencephalic aqueduct and is markedly phylogenetically conserved across the vertebrate species, from the lamprey fishes, amphibians, reptiles, birds, right through to mammals (Pezalla, 1983; Ten Donkelaar and de Boer-van Huizen, 1987; Fiebig, 1988; Kittelberger et al., 2006; Olson et al., 2017). In mammals, the PAG comprises a relatively large group of neurons (Liu and Hamilton, 1980; Behbehani, 1995) extending from the level of the posterior commissure rostrally, to the caudal inferior colliculi. Fibres of the mesencephalic trigeminal tract and tectospinal fibres, originating in the deep layers of the superior colliculus, form the lateral borders of the PAG. In the cat, the PAG is approximately 8 mm long and 2–3 mm wide (Subramanian et al., 2008), whilst the human PAG is approximately 14 mm long and 4–5 mm wide (Duvernoy, 1995) (Fig. 1).

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**Fig. 1.** [Location of the PAG] {Representation of the location of the PAG within the cat brain (left) and human brain (right), with three axial slices and the subdivisions of the PAG displayed. The PAG is depicted to almost surround the cerebral aqueduct in both the cat and human illustrations (red in colour figures). Note the animal orientation of the PAG (with dorsal towards the top of the page), and the human orientation (with dorsal towards the bottom of the page) to conform with animal PAG literature and human brain imaging literature. Abbreviations, IC = inferior colliculus; SCP = superior cerebellar peduncle; SN = substantia nigra; ICN = intercollicular nucleus; RN = red nucleus; SC = superior colliculus; vl, ventrolateral PAG, l, lateral PAG; dl, dorsolateral PAG; dm, dorsomedial PAG.}.

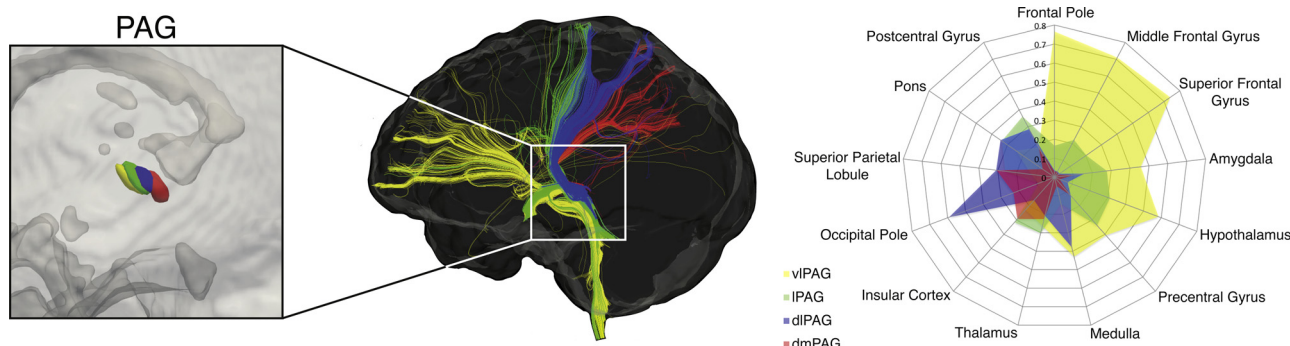


**Fig. 2.** [Connections and columnar structure of the PAG columns from animal tracer studies] {Anatomical afferent (top) and efferent (bottom) connections of the subdivisions of the periaqueductal gray matter from research in animals. The three-dimensional PAG columnar structure is represented in the animal orientation. Abbreviation, vl = ventrolateral; l = lateral; dl = dorsolateral; dm = dorsomedial. Adapted from Carrive et al., 1993 and Subramanian and Holstege, 2014.

Within the PAG, animal studies have demonstrated that no clear cytoarchitectonic boundaries exist between potential subdivisions of this nucleus (Behbehani, 1995; Holstege, 1991a). However, functional specificities of the PAG are expressed in the form of longitudinal columns along its rostro-caudal axis, comprising the dorsomedial (dmPAG), dorsolateral (dlPAG), lateral (lPAG) and ventrolateral (vlPAG) columns (Carrive, 1993) (Fig. 2). The discrimination of these columns has been proposed in both the rat and the cat, and evidence from both species has identified considerable rostrocaudal differences in the sizes and shapes of each of the columns (Subramanian et al., 2008; Subramanian, 2013; Subramanian and Holstege, 2011, 2013,

2014). The dorsolateral column, for example, is pronounced in its intermediate third, but very small in its caudal third, while the lateral and ventrolateral columns are still well developed in the caudal third (Carrive, 1993). An interesting question arises as to whether any systematic differences in PAG structure and function may exist across species, according to predominating characteristics tending towards ‘predator’ or ‘prey’ (Subramanian and Holstege, 2014). However, this question has not yet, to our knowledge, been investigated.

In humans, the post-mortem Duvernoy atlas of the human brainstem (Duvernoy, 1995) has allowed us to visualize similarities in the overall shape of the PAG to that reported in animals (represented in Fig. 1).



**Fig. 3.** [Connections of the PAG columns from human diffusion tractography] {Right, Radial diagram of relative connectivity of the clusters to predefined cortical targets, reproduced from Ezra et al. (2015). Left, Three-dimensional fibre tracking of cortical and subcortical projections from the four division of the human PAG to the cortex, excluding the cerebellum. Data is taken from one example subject from Ezra et al. (2015). Tractography demonstrates differential connectively patterns arising from the Dorsomedial (Red), Dorsolateral (Blue), Lateral (Green) and Ventrolateral (Yellow) aspects of the PAG. Image produced using DSI Studio.}.

However, with no inherent cytoarchitectural contrast between the columns, distinguishing sub-structures in the human PAG remains a challenge. As the animal PAG has been differentiated into columns using both functional information (Carrive, 1993) and the structural connections between PAG columns and the wider cortex (via tract tracing) (Holstege, 1991a), one potential non-invasive technique to subdivide the human PAG is that of diffusion tractography. This technique allows the probabilistic mapping of ‘macro’ scale cortical connections, by taking brain images that are able to assess the diffusion of water along structures such as white matter tracts across the cortex. Using this technique, recent work by Ezra and colleagues (Ezra et al., 2015) identified a similar longitudinal columnar structure by clustering the brain-wide connectivity profiles for each of the measured 3-dimension pixels (termed ‘voxels’) in the PAG (Fig. 3). In contrast to the animal PAG, both the lPAG and vIPAG columns appear to be larger towards the superior / rostral end, tapering within the inferior / caudal PAG, whilst the dIPAG and dmPAG appear to be slightly more homogenous along their length, and thus comparatively larger towards the inferior / caudal portion of the PAG (Ezra et al., 2015). Therefore, whilst this non-invasive technique cannot identify direct neuronal connections in a similar manner to tract tracing studies in animals, preliminary evidence of PAG substructures in humans appears to conform to the animal model of four longitudinal columns either side of the aqueduct, with possible differences in the size and shape of these columns.

### 1.2. Overview of PAG function in animals and humans

The PAG has been implicated across a broad range of physiological and behavioural functions, including cardiovascular, respiratory, locomotor and pain responses (to name a few) (Subramanian, 2013; De Oca et al., 1998; Mobbs et al., 2007; Pereira et al., 2010; Tracey et al., 2002; Benarroch, 2012; Paterson, 2013). Encompassing this spectrum of functionality is the over-arching theory that the PAG may both be involved in the integration of a multitude of sensory signals from the periphery, acting as a control centre for behavioural modulation as part of an integrated defense system. This theory postulates that the lPAG and dIPAG may orchestrate ‘active’ responses (such as fight or flight responses) when a threat is perceived as escapable (Carrive, 1993; Bandler and Carrive, 1988; Depaulis et al., 1992; Keay and Bandler, 2001; Yardley and Hilton, 1986). Conversely, the vIPAG is thought to be involved with ‘freezing’ type behaviours resulting from inescapable threats (Carrive and Bandler, 1991; Keay et al., 1997; Lovick, 1993; Tovote et al., 2016), including conditioned anticipation of breathlessness in humans (Faull et al., 2016; Faull and Pattinson, 2017).

It is important to explicitly note here that the proposed functionality of the collective PAG columns lies across two domains: 1) As a sensory information integrator, and 2) As an autonomic behavioural control

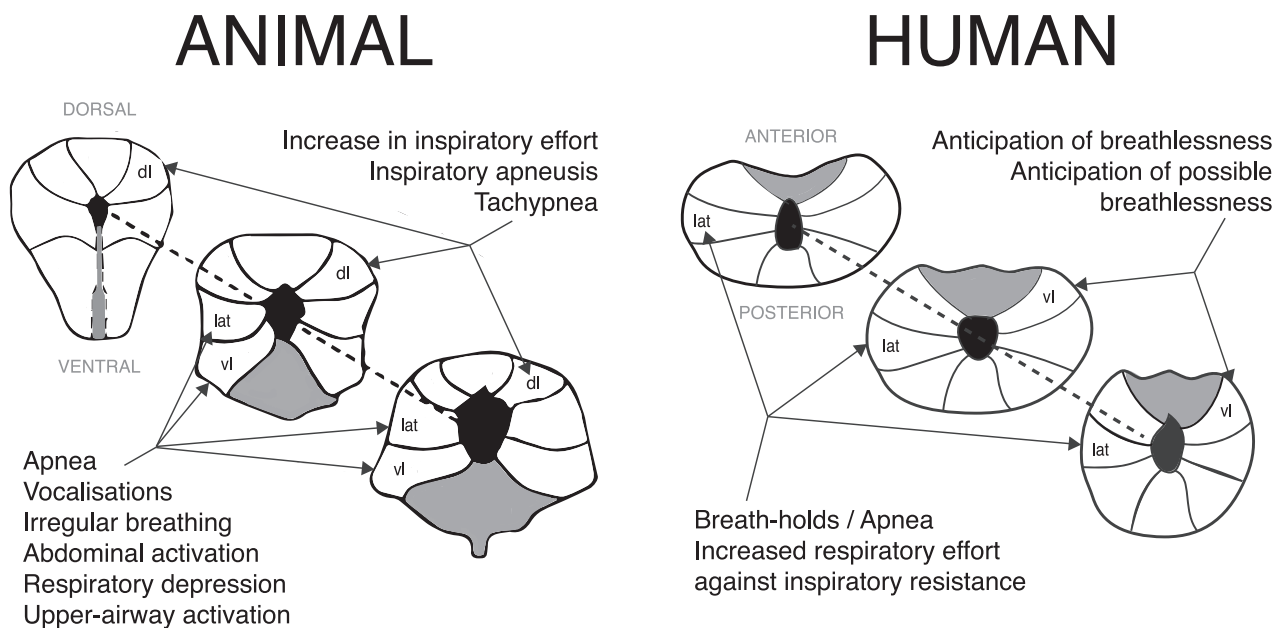
centre. Furthermore, the types of experiments conducted on the PAG can be largely classified into two discrete categories: 1) Perturbation studies, which allow us to probe the potentially causal relationships between excitability of PAG neurons and the resulting changes in physiology and behavior, and 2) Descriptive studies, whereby activity in the PAG is passively recorded and thus interpretation is limited to correlations with the integration of incoming sensation or resulting behavioural outcomes. In this review, we will attempt to highlight where the methods employed allow us to infer causality and control, and where we are limited to correlation and interpretation based largely on the integration properties of the PAG.

### 1.3. Respiratory functions of the PAG, animal studies

Animal models readily allow us to investigate the role of the PAG as an autonomic behavioural control centre in the brain, via direct and causal stimulation of targeted neurons. As such, the specific role of the PAG in animal respiratory control has been suggested since the 1930’s (Kabat, 1935). Kabat (1935) initially found that electrical stimulation of the PAG (then considered as one entity) in the cat could evoke an increase in the rate of respiration, changes in the amplitude or depth of breathing, and also a defensive ‘spitting’ response. Further investigations demonstrated that facio-vocal activity could also be evoked via electrical stimulation of the PAG (Kelly, 1946). However, owing to the non-specificity of electrical stimulation with regards to underlying neurons (Subramanian and Holstege, 2013), Huang et al. (2000) then utilised site-specific chemical stimulation (via a glutamate excitatory amino acid agonist) to demonstrate that explicit excitatory respiratory responses could be produced by stimulation of the dorsal PAG in the anaesthetised rat. One potential caveat to this approach is that studies on anaesthetised animals can also be problematic, whereby administration of anaesthetic drugs such as isoflurane are known to inhibit neurotransmission in key homeostatic and respiratory brainstem nuclei, such as the nucleus tractus solitarius, via complex presynaptic and postsynaptic mechanisms (Peters et al., 2008).

In an attempt to circumvent potential problems when investigating breathing in anaesthetized animals, Subramanian et al. (2008) have since used a decerebrate cat model to provide a detailed description of how distinct regions of the PAG produce specific respiratory responses (Fig. 4). For example, direct stimulation of the cat caudal vIPAG resulted in irregular breathing, whilst the dmPAG produced slow, deep breathing (Subramanian et al., 2008). These causal respiratory effects were then replicated in the anaesthetized rat, elucidating comparative physiology across higher order mammalian animal species (Subramanian, 2013; Subramanian and Holstege, 2013, 2011). Furthermore, these induced respiratory effects (particularly by the dorsal PAG) have also been replicated in the *in situ* rat preparation (Farmer et al., 2014).





**Fig. 4.** [PAG respiratory modulations in animals and humans] {The different types of respiratory modulations that have been evoked by direct stimulation in animal models (left - adapted from [Subramanian and Holstege \(2014\)](#)), and those observed during fMRI in humans (right). Note the animal orientation of the PAG (with dorsal towards the top of the page), and the human orientation (with dorsal towards the bottom of the page), and moving caudally/inferiorly in the PAG slices towards the right of the diagrams, to conform with animal PAG literature and human brain imaging literature.}.

#### 1.4. Respiratory functions in the PAG, human studies

Care must be taken when attempting to translate animal findings into human experiments for a number of reasons. Whilst animal models allow detailed investigation of the neuroanatomy and neurocircuitry of the PAG and its function as a control centre, they are also limited by both their invasive nature and their restricted behavioural interpretations, whereby subjective feedback is impossible. In contrast, human investigations are limited by both the correlational nature and the resolution constraints of non-invasive neuroimaging. An explicitly important note is that non-invasive investigations of human respiratory control often employ a reversed model to animal experiments, whereby ventilatory tasks are conducted and brain activation is measured, allowing only for correlations between brain activity and behaviour. Alternatively, small populations of patients with chronic movement disorders or pain can undergo stereotaxic electrode placement in the PAG, although the electrode location is restricted to dorsal or ventral PAG placement ([Green and Paterson, 2008](#)). These electrodes can be either stimulating or recording devices, allowing greater flexibility towards causal mechanisms in a direct stimulation setting. Although investigating the columns of the PAG in humans remains a challenge, the preliminary evidence suggesting the PAG to be potentially involved in respiratory integration (via neuroimaging) and control (via deep brain stimulation) warrant discussion.

Firstly, stereotaxic electrodes have been used to both stimulate and record from the human PAG. [Hyam et al. \(2012\)](#) produced increases in peak expiratory flow rate via stimulation of the PAG and subthalamic nucleus, whilst comparative stimulation of control nuclei in the sensory thalamus and globus pallidus interna did not evoke these respiratory changes. These results support work in animals ([Haxhiu et al., 2002](#)), whereby stimulation of the vlPAG induced airway smooth muscle relaxation in ferrets. In contrast, [Green et al. \(Green and Paterson, 2008; Green et al., 2007\)](#) used deep brain electrodes to record local field potentials in the PAG, substantia nigra or globus pallidus interna while patients underwent the task of imagining exercise. Imagining exercise evoked profound physiological responses, including increased respiratory rate, heart rate and blood pressure compared to rest. In conjunction with these cardiorespiratory changes, the greatest associated

neural increases were seen in the PAG (43% activation increase compared to rest), which increased to 87% above rest when the patients completed physical exercise on a cycle ergometer. Therefore, while deep brain electrode studies in humans only occur in patients with existing pathology, and cannot accurately discriminate activity between the subdivisions of the PAG, preliminary PAG stimulation studies support the work in animal models for a possible tier of respiratory control residing within the PAG. Additionally, electrode recording studies in patients have also identified activity in the PAG to be related to the integration of cardiorespiratory changes in the body, and thus provide evidence of an association (but not cause) with human PAG activity.

A variety of non-invasive imaging techniques also exist to examine brain structure and function. Functional MRI (fMRI) manipulates magnetic properties of blood within tissues to approximate brain function, which can then be indirectly associated with changes in respiratory function. These brain activity changes are measured within voxels, and when larger static magnetic fields are used, more signal within the brain tissue allows for finer resolution and smaller voxels to better differentiate small brain structures. With standard available scanner strengths ranging from 1.5 to 3–4 Tesla (T), the voxel resolutions for these brain imaging studies have been typically around 3 × 3 × 3 mm or above. These resolutions can become problematic for studying the PAG as it becomes blurred with surrounding structures, which can then be compounded by prominent physiological noise caused by both the adjacent pulsating aqueduct and breathing-induced artefacts from the chest ([Brooks et al., 2013](#)). However, at these field strengths and scanning resolutions, some midbrain activity has been identified (alongside widespread cortical activity) during maximal inspirations ([Topolovec et al., 2004](#)), resistive inspiratory loading ([Gozal et al., 1995; Hayen et al., 2017](#)), breath holding ([Pattinson et al., 2009](#)) and during hypercapnic-stimulated breathing (using positron emission tomography) ([Corfield et al., 2005; Brannan et al., 2001](#)). As previously noted, the interpretations of the midbrain results in these studies are limited by their spatial resolution constraints, whereby localization of activity to the PAG itself, and / or identification of differential activity across PAG columns is highly challenging.

With the recent introduction of 7 T MRI, functional scanning resolution can now be improved to allow potential differentiation of small

nuclei such as the columns of the PAG. Using simple breath holds, 7 T imaging has revealed localised brain activity along the IPAG column in humans, utilising 1 x 1 x 1 mm voxels (Faull et al., 2015). Following this, two separate studies demonstrated that functional activity could be differentiated to the IPAG during breathlessness (induced by inspiratory resistive loading), and to the vIPAG during anticipation of these loads (Faull et al., 2016; Faull and Pattinson, 2017). Therefore, whilst still correlational in nature, the opportunity to non-invasively investigate the integration of respiratory sensory information within the PAG columns in humans now appears to be within the realms of possibility, as the advent of ultra-high field neuroimaging becomes more commonplace (Faull et al., 2016; Faull and Pattinson, 2017; Faull et al., 2015; Satpute et al., 2013).

### 1.5. Investigating direct descending respiratory control circuitry in animals

Initial causal investigations into descending respiratory motor control pathways from the PAG were primarily focused on dorsal PAG-induced tachypnea, or rapid breathing. Using micro-injection of homocysteic acid in the dorsal PAG of the rat, marked changes in respiratory rhythms were observed. These changes included increases in respiratory frequency and diaphragmatic activity, and increases in inspiratory neuronal firing within the nucleus tractus solitarius (Huang et al., 2000; Subramanian et al., 2007). These striking respiratory behaviours were then attenuated by the injection of a beta-adrenergic antagonist (propranolol) into the nucleus tractus solitarius, revealing an important modulatory relationship and leading to speculation of a possibility noradrenergic connection between the two nuclei (Huang et al., 2000; Subramanian et al., 2007). Dorsal PAG-evoked tachypnea was also attenuated via bilateral inhibition of the lateral parabrachial nucleus (Hayward et al., 2004), demonstrating a further link in the pathway of dorsal-PAG evoked respiratory responses.

In addition to direct excitation of the dorsal PAG inducing tachypnea, disinhibition of the dorsal PAG (using a GABA<sub>A</sub>-receptor antagonist) was also found to produce similar, dose-dependent respiratory tachypnea (Hayward et al., 2003). These results suggest that GABA<sub>A</sub> may also play a role in mediating dorsal PAG neurons during respiratory defensive behaviours. Zhang et al. (2009) further showed that both activation and disinhibition of the dorsal PAG not only increases respiratory drive, but also reduces the volume-timing reflex sensitivity, i.e. the Hering-Breuer reflex, allowing for greater volume changes during inspiration and expiration before phase-switching occurs (Zhang et al., 2009).

Alongside the nucleus tractus solitarius and the lateral parabrachial nucleus, the behavioural breathing circuitry of the PAG is also likely to involve key nuclei in the hypothalamus (Horiuchi et al., 2009; Ryan and Waldrop, 1995). Direct connections have been identified between the dorsal PAG and the caudal, dorsomedial and posterior hypothalamic nuclei (Horiuchi et al., 2009; Ryan and Waldrop, 1995; Cameron et al., 1995; Vertes and Crane, 1996), and the dorsal PAG appears to be directly influenced by the hypothalamus for integrated control of breathing (Dampney et al., 2008; Horn and Waldrop, 1998). For instance, respiratory outputs of the dorsal PAG can be virtually abolished by inhibition of the dorsomedial hypothalamus, while the reverse interaction was not found (Horiuchi et al., 2009). The dorsomedial hypothalamus also has a well-documented role in the pathway between the suprachiasmatic nucleus and the ventrolateral preoptic area for control of the transition between sleep and wakeful states (Chou et al., 2002), and the relationship between the dorsomedial hypothalamus and PAG may support the observation that a subset of PAG cells correlate with respiratory patterns in a sleep state-dependent manner (Harper et al., 1991). Therefore, it appears likely that the respiratory integration and control functions of the PAG act within a network that also incorporates key respiratory hypothalamic nuclei, allowing governance of ventilation according to states of consciousness and activity.

Moving beyond considering only dorsal PAG-induced tachypnea or

collective PAG activity, induction of tachypnea was found to be localised to areas within the dIPAG and IPAG in the decerebrate cat, whilst the stimulation of the dmPAG produced mild decreases in respiratory frequency (Subramanian et al., 2008). The localisation of tachypnea in the rat PAG was also found to be predominantly within the dIPAG, rather than the dmPAG or the IPAG (Huang et al., 2000; Iigaya et al., 2010; Subramanian, 2013; Subramanian and Holstege, 2013). These studies provide direct electrophysiological evidence of how these PAG columns modulate medullary respiratory circuits to produce specific respiratory responses such as tachypnea, inspiratory apnoeas and breath-holds in the rat. To induce these effects, the PAG appears to modulate three types of respiratory neurons in the caudal medulla, the late-inspiratory (late-I) and the post-inspiratory (post-I) neurons of the Bötzinger complex, and the pre-inspiratory (pre-I) neurons of the pre-Bötzinger complex. In particular, the PAG control of the late-I and post-I cells contributes to the conversion of eupnea to a behavioural breathing pattern (Subramanian, 2013). For example, the vIPAG produces laryngeal breath-hold (a typical autonomic expression of fear) by tonic activation of the post-I neurons while simultaneously inhibiting the late-I cells. Conversely, during stimulation of the IPAG, prolonged inspiration is induced via an excitation of late-I neurones alongside inhibition of post-I neurones, while dIPAG-induced tachypnea is induced via simultaneously balanced discharge of the late-I and post-I neurons. Therefore, by either phasically or tonically activating or inhibiting medullary respiratory circuits (Subramanian, 2013; Subramanian and Holstege, 2013), the PAG columns appear to have the descending control network required for behavioural expression of breathing, such as during avoidance, fight, flight, or freezing (Subramanian et al., 2008; Subramanian and Holstege, 2014).

### 1.6. Integrating ascending respiratory information for coordinating respiratory behaviours in animals

Defensive behaviours such as fight or flight are highly conserved throughout phylogeny, and the PAG is thought to be a key nucleus in both integrating sensory information and coordinating defensive behaviours within the brain's threat circuitry (De Oca et al., 1998; Mobbs et al., 2007; Pereira et al., 2010; Tracey et al., 2002; Benarroch, 2012). These behaviours can be triggered by any number of threatening situations, and specific respiratory stressors may include situations including hypoxia (reduced blood oxygen), hypercapnia (elevated blood carbon dioxide), or restricted or labored breathing (as one type of breathlessness). One important mechanism that would allow for sensory integration and subsequent respiratory control in the PAG would be chemoreception.

Chemoreception broadly encompasses the monitoring of partial pressures of blood gases, allowing for direct comparisons between ventilation and the requirements of the periphery (Ballantyne and Scheid, 2001). Although the PAG has not been identified as a chemoreceptive site itself, such as seen in the retro-trapezoid nucleus, medullary raphe, nucleus tractus solitarius, locus coeruleus and pre-Bötzinger complex (Kuwaki et al., 2010), c-Fos labelling in all columns of the PAG has been observed in association with activity in arterial chemoreceptors in anaesthetised rats (Hayward and Reitzenstein, 2002). Whilst these results do not directly infer PAG-induced respiratory control as a result of chemoreception, work performed in non-anesthetized, supracollicularly decerebrated, paralyzed and ventilated cats has shown that both hypercapnia and carotid sinus nerve stimulation could convert tonically firing neurons in the PAG to phasic rhythmic activation during late inspiration and post-inspiration (Chen et al., 1991). As such, it has been suggested that an anoxia-sensitive suffocation alarm system may exist within the PAG (Schmitel et al., 2012), although only lesions to the dorsal PAG appear to augment the hypoxic ventilatory response (Lopes et al., 2014). Therefore, although it remains to be determined whether the PAG functions as a primary chemoreceptive site, these studies suggest that it may be involved in the

integration of chemoreceptive information and subsequent behavioural breathing modulation, potentially playing a continuous role in the subconscious homeostatic monitoring and control of ventilation.

### 1.7. Integration within a wider respiratory network in animals and humans

As sentient beings our breathing encompasses more than respiratory-related reflexes, and fine tuning of respiratory behaviours is often necessary for higher-order functions. Ventilatory modifications can be made during phonation or stress, and as humans, voluntary changes to our breathing can be largely called upon whenever desired, such as whilst scuba diving or practicing yoga. These changes indicate the presence of a conscious and integrated ventilatory control network, and here we will discuss the relationship between the PAG and cortical brain regions that have been identified as potential modulators of respiration.

An outline of the existing animal evidence for the anatomical cortical connections of the columns of the PAG has been previously provided by Dampney et al. (2013) (summarised in Fig. 2). Firstly, the primary motor cortex has been demonstrated to directly project to within the vicinity of spinal respiratory motor neurons (Rikard-Bell et al., 1985), allowing for possible voluntary control over diaphragm and thoracic respiratory muscles. Cortical connections have also been identified from the trunk area of the primary motor cortex to both the IPAG and vIPAG (Dampney et al., 2013), demonstrating the potential anatomical scaffolding that would allow for direct input from respiratory-related areas of the motor cortex into the PAG. Meanwhile, conscious ventilatory sensations have been traditionally thought to include both sensory thoracic proprioception and affective emotional evaluation (Davenport and Vovk, 2009). Interestingly, whilst there is no evidence of monosynaptic anatomical connections between the primary sensory cortex and the PAG, rich PAG inputs have been identified from cortical limbic areas involved in emotional evaluation, such as the prefrontal cortex and amygdala (Tovote et al., 2016; Beitz, 1982; Gabbott et al., 2005; Rizvi et al., 1991; Holstege, 1991b). Therefore, it is possible that the PAG is directly involved with both respiratory motor commands and the emotional evaluation of respiratory sensations, although these findings are drawn from anatomical investigations (Holstege, 2014), rather than functionally-mediated connections between structures.

Again, whilst the ability to non-invasively investigate the connections of the PAG is somewhat limited in humans, one previously-mentioned paper has revealed preliminary insight into the ‘macro’ structural connections of the human PAG columns using diffusion tractography (Ezra et al., 2015). In this work, Ezra et al. (2015) used subject-specific clusters within the PAG to quantify the structural connectivity to pre-defined cortical and subcortical areas. In doing so, they identified predominant fronto-limbic connectivity of the vIPAG, in comparison to principally sensorimotor connectivity of the IPAG/dIPAG (Fig. 3). However, it must also be noted here that diffusion tractography cannot encapsulate the direction of connections between structures, and can rather be considered as a non-directional probabilistic map of potential existing underlying anatomical connections via axon bundles. Whilst some of the revealed connectivity patterns were consistent with animal tracer studies, the authors noted that the prefrontal cortex connections were principally targeted to the vIPAG, whereas animal studies have also demonstrated distinct connections to IPAG and dIPAG from the prefrontal cortex (Floyd et al., 2000; An et al., 1998). These discrepancies may be due to anatomical differences between species, or conversely, subtle crossing tracts that may be masked by the resolution constraints when using diffusion tractography to differentiate white matter projections between small, densely packed PAG columns.

An alternative measure of connectivity between brain areas is via the technique of ‘functional connectivity’ – a measure of the temporal synchronicity of brain activity in remote regions of cortex. Functional connectivity is thus thought to reflect temporal coherence and

functional communication of neuronal activity between anatomically distinct brain areas (Gerstein and Perkel, 1969; Van Den Heuvel and Pol, 2010). Therefore, functional connectivity measures can potentially expose indirect, task-specific connections between regions that may not be structurally connected, but again mainly in a correlational (rather than causal) manner in human studies. Using this technique, recent evidence has revealed IPAG connectivity to sensorimotor structures at rest, and increased synchronicity to the amygdala during inspiratory resistive loading (Faull and Pattinson, 2017). Conversely, the vIPAG showed fronto-limbic connectivity at rest, and increased connectivity with the insula during resistive loading. Furthermore, the vIPAG appeared to *disconnect* (or reduce its temporal synchronicity) with the IPAG and sensorimotor structures during conditioned anticipation of resistive loading, consistent with behavioural ‘freeze’ responses observed in animals when facing an inescapable threat (De Oca et al., 1998; Keay and Bandler, 2001; Bandler et al., 2000; Bandler and Shipley, 1994).

Therefore, both animal and human investigations have revealed hugely rich structural and functional connectivity patterns between the PAG columns and widespread sensorimotor and limbic brain regions. The position, connectivity and respiratory functions of the PAG make it an ideal candidate for a reflexive and integrated behavioural modulator of breathing, particularly during times of altered homeostasis or perceived threat. Due to the necessity of maintained respiration for our continued existence, and the key role of the PAG in communicating between autonomic brainstem and conscious cortical respiratory regions, we will now explore the particular importance of the PAG columns in the threat of breathlessness.

### 1.8. The PAG and the interoceptive threat of breathlessness in survival

Breathlessness is a debilitating and threatening perception, and it relies on our ability to interoceptively monitor the state of our breathing. Breathlessness encumbers countless sufferers of chronic obstructive pulmonary disease (COPD), asthma, cardiovascular disease, neuromuscular diseases, cancer and panic disorder. Discrepancies between breathlessness severity and objective measures of lung function are well known and remain unexplained (Herigstad et al., 2011; Hayen et al., 2013; Lansing et al., 2009). These discrepancies are commonly rationalised in terms of inadequate measures of physiology, such as abnormal (but unmeasured) thoracic muscle function, or inaccurate lung function tests. However, an alternative explanation relates to variability within the brain’s processing of respiratory sensations (Herigstad et al., 2015, 2017), with the PAG likely to play a key role due to its position and function within both ventilatory control and threat behavior circuits.

Understanding the mechanisms by which the brain interprets bodily sensations to form a conscious perception is in its infancy. While perceptual systems have traditionally been considered to provide a veridical representation of sensory stimuli, this cannot explain the prominent presence of symptom discordance, such as that reported with breathlessness (Herigstad et al., 2011; Hayen et al., 2013; Lansing et al., 2009; Herigstad et al., 2017). Therefore, recent interoceptive theories have proposed a more comprehensive, predictive Bayesian model of symptom perception (Seth, 2013; Barrett et al., 2015; Van den Bergh et al., 2017). This model includes a set of expectations, or ‘priors’, which are combined with incoming sensory information from the body, and reconciling these expectations and sensations thus form conscious perception. Importantly, prediction errors arise when incoming sensory information does not match prior expectations, and these errors are thought to stimulate learning via updating priors and improving the efficiency of the model (Barrett et al., 2015). The PAG (and in particular the vIPAG (McNally and Cole, 2006)) has been identified in both animals and humans to possibly encode these prediction errors (Roy et al., 2014; McNally et al., 2011) in a manner that makes sound teleological sense, residing alongside its role as an interface between autonomic and



conscious threat processing.

Traditionally, breathlessness has been considered as a multi-dimensional and multifaceted perception, thought to be induced by the mismatch of information between afferent respiratory signals and efferent ventilatory drive (almost akin to prediction error) (Schwartzstein et al., 1989, 1990). Whilst conventional views have often classified these sensory dimensions into broad categories such as ‘work of breathing’ from respiratory muscles in the torso, ‘air hunger’ from chemoreceptive signals in the blood, and ‘chest tightness’ from airway obstruction or restriction (to name a few (Lansing et al., 2009; Laviolette and Lavenexiana, 2014)), these boundaries might not sufficiently encapsulate the complexity of individual breathlessness perceptions. Each individual brings their own set of prior expectations, interoceptive abilities and bodily awareness (Garfinkel et al., 2015, 2016), and thus ‘breathlessness’ will be both vastly quantitatively and qualitatively different between individuals. This perceptual density is reflected in the widespread associated brain activity reported in limbic, prefrontal and sensorimotor cortices in human breathlessness research (see previous reviews (Herigstad et al., 2011; Hayen et al., 2013)).

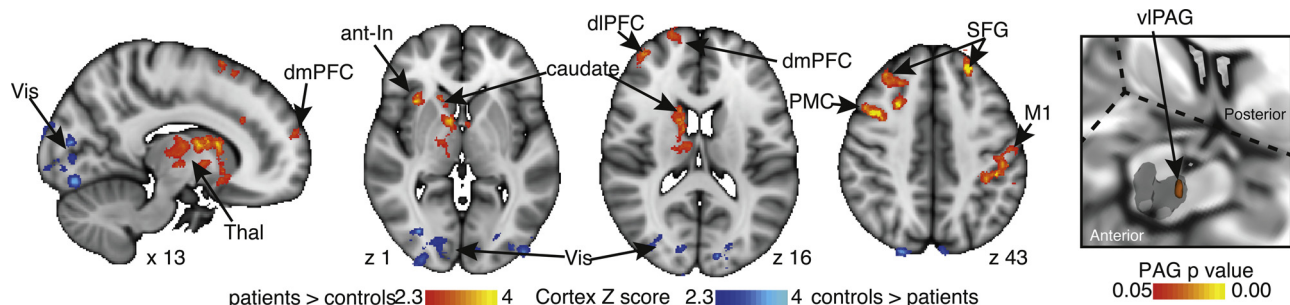
To more generically investigate the multiple dimensions of breathlessness, we can independently explore two important and distinct components: 1) Conditioned anticipation of breathlessness, and 2) Interoceptive perceptions of respiratory sensations during the event. These components represent a behavioural divide between a conditioned, future-oriented emotional state (anticipation), typically associated with worry, apprehension and ‘freezing’ behaviours in animal homologues (and where prior expectations dominate), and a multi-modal, fear-encoding state during the internal perception of breathlessness, associated with more active ‘fight/flight’ behaviors (Schroijen et al., 2016) and encompassing a dynamic interplay between predictions and sensory information. Importantly, the anticipatory anxiety of breathlessness can induce avoidance of everyday activities (Herigstad et al., 2011; Hayen et al., 2013; Janssens et al., 2011), and is thus intricately linked with disease burden in clinical populations (Solano et al., 2006; Smoller et al., 1996).

As previously mentioned, recent work has now identified differential columnar PAG activity associated with both anticipation and perception of breathlessness in humans (Faull et al., 2016; Faull and Pattinson, 2017). Using an aversive inspiratory resistance, healthy volunteers were conditioned to associate a previously neutral shape with upcoming resistive loading. vIPAG activity was observed during cued aversive anticipation of resistive loading, which was reduced (alongside reported breathlessness intensity and anxiety) when the contingency pairings during learning were lowered from 100% to 50% (Faull et al., 2016). These results indicate a potential role for the vIPAG during aversive anticipation of breathlessness, where a passive response to an inescapable threat is reduced when the predictive cue is weaker.

Comparatively, activity in the IPAG was observed during resistive loading itself (Faull et al., 2016), with a subsequent study revealing scaled activity in the IPAG with behavioural ratings of breathlessness intensity (Faull and Pattinson, 2017). Therefore, it appears that activity in the IPAG during the actual perception of breathlessness may correspond to the hypothesised ‘active’ threat response associated with the IPAG/dIPAG.

The PAG is not presumed to work in isolation, and the extensive cortical activity identified in a host of previous breathlessness research exposes the potential extent of this breathlessness network (Faull et al., 2016; Faull and Pattinson, 2017; Hayen et al., 2017; Pattinson et al., 2009; Brannan et al., 2001; Davenport and Vovk, 2009; Herigstad et al., 2015, 2017; Banzett et al., 2000; Evans et al., 2002; Stoeckel et al., 2016; Liotti et al., 2001; McKay et al., 2008; Von Leupoldt et al., 2009a, b; Pattinson and Johnson, 2014). Whilst modern neuroimaging techniques allow us to envision cortical activity in primary sensory cortices relevant for initial signal transduction of afferent inputs, we must tread carefully in labelling this ‘conscious perception’ or ‘breathlessness’. Instead, breathlessness is likely to be an evolving function embedded within dynamic brain networks, where transduced sensory inputs are continuously compared to the brain’s expected sensations and model of the world (Seth, 2013; Barrett et al., 2015; Van den Bergh et al., 2017; Stephan et al., 2016). Thus, linear increases in activity within explicit cortical areas are not likely to produce corresponding linear changes in breathlessness, and we need to equip ourselves with appropriate computational strategies (Stephan et al., 2016; Petzschner et al., 2017) that can bring us closer to tackling these more difficult, more multi-dimensional brain network models in the future, encompassing both the PAG and wider cortex.

To highlight its clinical relevance, breathlessness is considered the main symptom of COPD, and is even a better predictor of mortality in these patients than measures of lung function (Celli et al., 2004). It is well known that system moderators (such as anxiety and depression) can alter perception of breathing sensations (Spinoven et al., 1997), and individuals with COPD have a prominent presence of psychological co-morbidities, including anxiety and panic disorder (Smoller et al., 1996; Gretchen and Brenes, 2003; Giardino et al., 2010). As breathlessness can be both a cause and a symptom of distress (for example as a manifestation of anxiety or panic-like symptoms), a worsening cycle of symptomology and discordance can be provoked in those with chronic breathlessness exposure. To consider the potential role of the PAG in this symptom profile, we ran a targeted PAG analysis on a group of COPD patients compared to matched controls (previously published data (Herigstad et al., 2015)), and found greater PAG activity when viewing breathlessness-related word-cues in the patients (Fig. 5). Interestingly, chronic exercise training can induce structural remodelling and a reduction in activity in cardiorespiratory areas of the animal



**Fig. 5.** [The PAG in COPD] {Targeted re-analysis of the PAG from published data (Herigstad et al., 2015), where 41 individuals with chronic obstructive pulmonary disease were compared with 40 age-matched healthy volunteers whilst viewing breathlessness-related word cues. The PAG is represented in the human orientation. Abbreviation, Vis = visual cortex; Thal = thalamus; dmPFC, dorsomedial prefrontal cortex; ant-In = anterior insula; caudate = caudate nucleus; dIPFC = dorsolateral prefrontal cortex; PMC = premotor cortex; SFG = superior frontal gyrus; M1 = primary motor cortex; vIPAG = ventrolateral periaqueductal gray. The images consist of a colour-rendered statistical map superimposed on a standard (MNI 1 x 1 x 1 mm) brain, and significant regions are displayed with a threshold  $Z > 2.3$ , with a cluster probability threshold of  $p < 0.05$  (corrected for multiple comparisons).}.



brain and brainstem (including the PAG) (Ichiyama et al., 2002; Nelson et al., 2005; Nelson and Iwamoto, 2006), and the most effective treatment for breathlessness in COPD is currently a course of exercise and education (pulmonary rehabilitation). Therefore, the PAG may be an important target for future research in both understanding and treating discordant, threatening breathlessness symptoms with chronic lung disease.

## 2. Conclusions and future directions

Whilst breathing is a vital survival and autonomic function, it is often at the mercy of higher conscious control and perception. Importantly, the PAG lies within an integrated respiratory network, bridging the gap between autonomic brainstem control and cortical motor and perception networks. Both its function and connections make the PAG an ideal candidate to play a key role in integrating, modulating and adapting ventilation when the need (or the reflexive response) arises. However, by considering the substructural columns of the PAG we can glean a more nuanced understanding of its role in ventilation and beyond, and we need to push to progress from considering this structure as a unitary entity.

One particularly salient form of respiratory threat is the perception of breathlessness. Breathlessness may arise when ventilatory drive does not match perceived respiratory needs, and is likely to vary vastly depending on both the context and the individual. The vPAG has been identified as a site for encoding prediction error within the Bayesian model of perception and interoception, and together with the columnar roles in breathing and integrated behavioural responses to threat, the PAG is revealed as an ideal candidate centre for discordant symptomatology in chronic breathlessness. High-resolution neuroimaging, together with advanced computational and electrophysiological strategies are needed to help investigate the role of the PAG columns within dynamic brain networks of breathlessness perception. These strategies have the potential to reveal sites of (mal)adaptation and thus potential treatment targets in those suffering from discordant, chronic breathlessness.

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